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<b>(21) International Application Number:</b> PCT/EP96/00373 <b>(22) International Filing Date:</b> 30 January 1996 (30.01.96) <b>(30) Priority Data:</b> 0108/95 31 January 1995 (31.01.95) DK <b>(71) Applicant (for all designated States except US):</b> NEUROSEARCH A/S [DK/DK]; Smedeland 26B, DK-2600 Glostrup (DK). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> MOLDT, Peter [DK/DK]; Langebjergvej 355, DK-3050 Humleback (DK). <b>(74) Agent:</b> GRÜNECKER, KINKELDEY, STOCKMAIR & SCHWANHÄUSSER; Maximilianstrasse 58, D-80538 München (DE).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> ASTAXANTHIN SUSPENSION  <b>(57) Abstract</b>  A suspension of astaxanthin in oil and use thereof in the manufacture of feed for aquatic animals.		

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## ASTAXANTHIN SUSPENSION

This invention relates to a stable oil suspension of astaxanthin with high bioavailability, and the use thereof.

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### PRIOR ART

Astaxanthin belongs to the carotenoids, which is a group of compounds exhibiting a wide range of yellow to red colour nuances. Carotenoids give rise to several of the colour characteristics of plants, and aquatic animals, for example.

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Carotenoids are insoluble in water and are only slightly fat soluble which means that solutions of carotenoids for direct application are unavailable. Furthermore, carotenoids are very sensible to oxidation.

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The colouring effect of crystalline carotenoids, which can be isolated in laboratories, is very low due to the insolubility and the particle size of the crystals.

In order to increase the colouring effect and the resorbability of carotenoids, it is necessary to reduce the particle size of the carotenoids and to protect the carotenoids from oxidation.

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Astaxanthin is used in fish-farming to impart a pink colour to shrimp, trout, salmon etc.

Currently, two gelatine-coated products (Carophyll Pink™) containing 5% and 8% astaxanthin, respectively, are commercially available. These products are given in the feed.

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These products, however, suffer from the disadvantages of having a reduced bio-availability due to a very slow dissolution rate of gelatine at low temperatures, and a limited storage time due to a hardening of the gelatine capsule; furthermore, there is a considerable waste of the products (approximately 15%) during production of feed by extrusion.

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**OBJECTS OF THE INVENTION**

It is an object of the invention to provide a stable suspension of astaxanthin with a high bioavailability even at low temperatures. Furthermore, it is an object of the invention to provide a product that can be added to the feed after extrusion and thereby obviating the loss of colour pigment in feed production.

Additional objects will be obvious to a person skilled in the art.

**SUMMARY OF THE INVENTION**

The invention comprises thus the following:

A suspension of astaxanthin in oil wherein a majority of the astaxanthin particles have a particle size less than 2  $\mu\text{m}$ ;

such a suspension wherein a majority of the astaxanthin particles have a particle size less than 1  $\mu\text{m}$ ;

such a suspension wherein the concentration of astaxanthin is between 0.03% and 50%;

such a suspension wherein the concentration of astaxanthin is between 5% and 20%;

such a suspension containing an antioxidant;

the use of such a suspension in the manufacture of feed for aquatic animals;

and such use whereby the oil suspension is absorbed into the feed after extrusion.

The suspension according to the invention can be prepared by milling of astaxanthin, if desired in the presence of oil. Suitably, astaxanthin is milled without oil, whereafter oil is added and the milling continued, if desired.

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Oxidation of astaxanthin during the dry milling operation can be reduced by the application of an inert gas atmosphere.

Another method by which astaxanthin could be obtained in finely divided form is by abrupt precipitation from a solution of astaxanthin in a solvent.

The astaxanthin particles in the suspension of the invention have a particle size less than 2  $\mu\text{m}$ , especially less than 1  $\mu\text{m}$ . More preferred is an astaxanthin particle size less than 0.5  $\mu\text{m}$ , especially less than 0.1  $\mu\text{m}$ .

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The oil can be any animal or vegetable oil suitable for use in feed for aquatic animals. Such oil includes fish oil, such as for example mackerel oil, sprat oil and launce oil.

Antioxidants, such as for example tocopherols, especially  $\alpha$ -tocopherol, butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), ascorbylpalmitate or ethoxyquin, can be added to the suspension to avoid oxidation.

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Further stabilisation of the solution of the invention can be obtained by keeping the suspension at temperatures below the solidification temperature.

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Feed for aquatic animals suitably contains 15-30 % fat. Fat can be added by absorption of oil into the feed after extrusion, if desired using vacuum. The oil suspension of the invention could suitably be added to the feed using the same procedure, whereby an addition of fat and astaxanthin is obtained in one step.

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Preferably, the suspension of the invention has a high concentration of astaxanthin and is diluted with additional oil before use.

The final concentration of astaxanthin in fish feed is suitable between 25 and 100 ppm.

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**Detailed Description of the Invention**

The invention will now be described in greater detail with reference to the following example, which are given by way of illustration and are not to be construed as  
5 limiting.

**Milling of Astaxanthin**

- 10 Astaxanthin (0.1 g) is milled for one hour totally in a Fritsen planetary micro mill pulverisette 7, speed 6. 5 ml of Launce oil ( Esbjerg Fiskeindustri ) is added and milling is continued for 15 min, speed 1.5. Particle size is measured by microscopy and found to be less than 2  $\mu\text{m}$  for the majority of the particles.  
This suspension is poured on to a glass and placed in the dark at room temperature  
15 for 14 days.

The above procedure is repeated, and the suspension obtained is compared with the 14-day-old suspension.

- 20 The small particles show a tendency to agglomeration but nothing indicates that the particles grow when standing. There is no sedimentation of astaxanthin.

Launce oil solidifies at  $-18^{\circ}\text{C}$ .

**CLAIMS:**

1. A suspension of astaxanthin in oil wherein a majority of the astaxanthin particles have a particle size less than 2  $\mu\text{m}$ .
2. A suspension as in claim 1 wherein a majority of the astaxanthin particles have a particle size less than 1  $\mu\text{m}$ .
3. A suspension as in claim 1 wherein the concentration of astaxanthin is between 0.03% and 50%.
4. A suspension as in claim 3 wherein the concentration of astaxanthin is between 5% and 20%.
5. A suspension as in claim 1 containing an antioxidant.
6. Use of the suspension in claim 1 in the manufacture of feed for aquatic animals.
5. The use according to claim 6 whereby the oil suspension is absorbed into the feed after extrusion.

## INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/EP 96/00373A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A23K1/16 A23K1/18 A23L1/275

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A23K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 14645 (GIST-BROCADES N.V.) 5 August 1993 see page 4, line 3-11 see page 6, line 1 - page 7, line 2 see examples I-III see claims 1-6,8	1
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 96/00373

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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PCT/EP 96/00373

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